

The Plant Microbiome: From Ecology to Reductionism and Beyond

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Abstract

Methodological advances over the past two decades have propelled plant microbiome research, allowing the field to comprehensively test ideas proposed over a century ago and generate many new hypotheses. Studying the distribution of microbial taxa and genes across plant habitats has revealed the importance of various ecological and evolutionary forces shaping plant microbiota. In particular, selection imposed by plant habitats strongly shapes the diversity and composition of microbiota and leads to microbial adaptation associated with navigating the plant immune system and utilizing plant-derived resources. Reductionist approaches have demonstrated that the interaction between plant immunity and the plant microbiome is, in fact, bidirectional and that plants, microbiota, and the environment shape a complex chemical dialogue that collectively orchestrates the plant

microbiome. The next stage in plant microbiome research will require the integration of ecological and reductionist approaches to establish a general understanding of the assembly and function in both natural and managed environments.

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INTRODUCTION

The causes and consequences of plant-associated microbial variation have been the subject of intense study for over a century. Following the discovery that atmospheric nitrogen is fixed by bacteria residing in leguminous root nodules came the understanding that plants are associated with an abundance of diverse microbes. Hypotheses that arose in that period are fundamental to the field to this day. Among them are the notions articulated by Lorenz Hiltner (60): that plant-derived nutrients attract beneficial microbiota in a species-specific manner and that this mechanism is exposed to exploitation by pathogens.

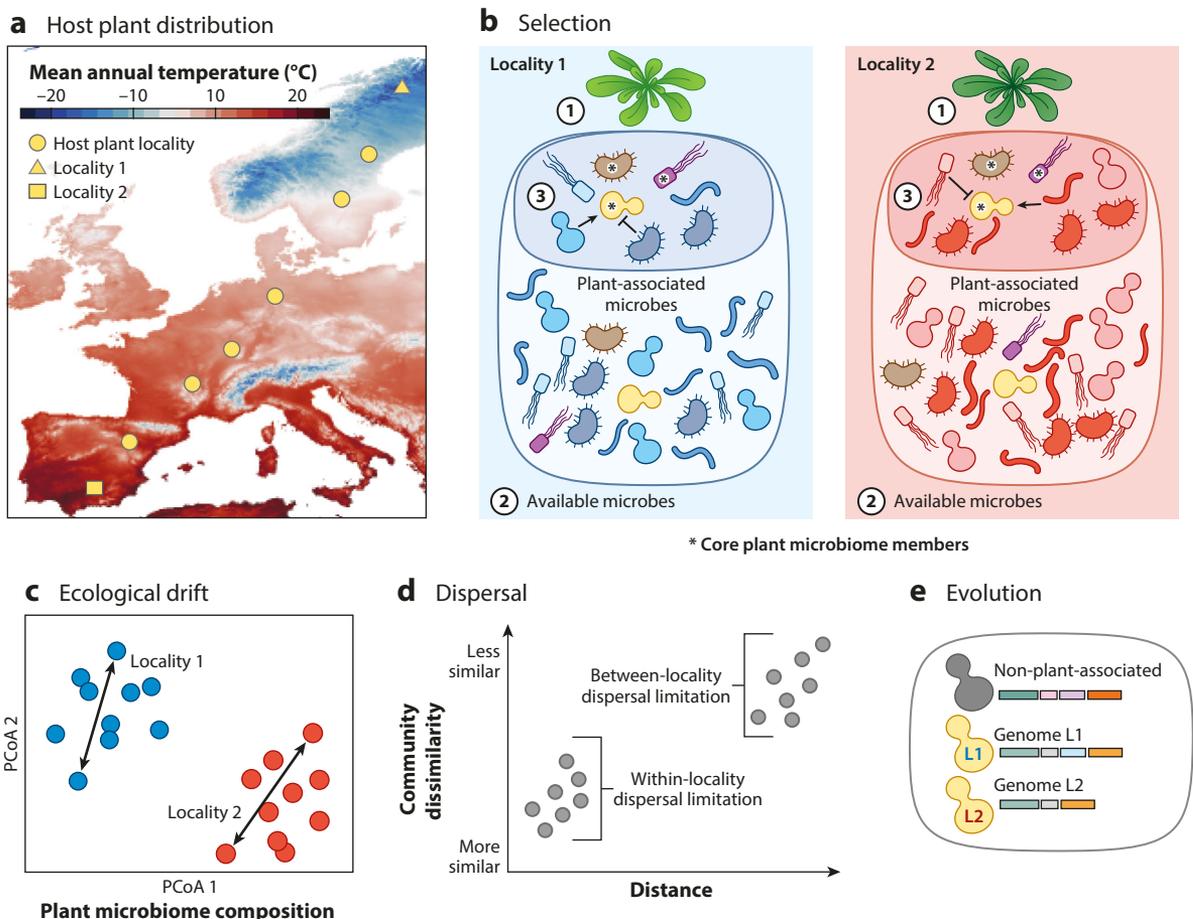
For over a century, the field relied on culture-dependent approaches to illuminate and study the multitude of plant microbiome inhabitants, which include fungi, bacteria, protists, and viruses. However, the stunning extent and distribution of this diversity revealed by culture-independent and high-throughput molecular approaches over the past two decades have had a transformative effect on our understanding, study, and application of plant-microbe research. Naturally, the reductionist study of plant-microbe interactions aimed at elucidating mechanisms cannot keep pace with the accelerating rate of ecological characterization of plant-associated microbial diversity. This calls for new strategies to merge the two approaches into a single framework in which census experiments (e.g., 16S amplicon and metagenomic censuses) can inform and prioritize reductionist studies of plant-microbe interactions. Here, we review the knowledge gained from census and comparative studies of the ecological and evolutionary processes shaping plant microbiota. Next, we review the mechanistic insight into the assembly of plant microbiota and their effects on plant immunity and development achieved by reductionist research. Finally, we attempt to synthesize these two approaches, following the belief that the full informative power of plant microbiome research for both basic and applied questions requires an approach that is both ecological and reductionist.

ECOLOGICAL AND EVOLUTIONARY PROCESSES SHAPING THE PLANT MICROBIOME

The era of culture-independent, high-throughput plant microbiome study began with an exploratory phase of the diversity and composition of microbial taxa and genes across plant habitats. Primarily based on census data, these studies confirmed the century-old hypothesis that plants harbor distinct microbiota, which represent a subset of those found in the ambient environment (15, 86). This led to the multistep model of plant microbiome assembly, whereby specific microbes in the environment are recruited to plant surfaces, followed by additional filtering as microbial taxa colonize the interior of plant organs (16, 38). Such large effects of plant habitats on microbial communities led to the hypothesis that plant-associated microorganisms are adapted to the unique environments provided by their plant hosts. To date, studies largely conform to this paradigm, but they have revealed that additional ecological and evolutionary processes can strongly shape plant microbiota (Figure 1).

Ecological Factors Shaping Plant Microbial Diversity and Composition

Selection by plant and environmental factors of particular microbial taxa plays a large role in the assembly of plant microbiota; however, additional ecological processes can influence community



(Caption appears on following page)

Figure 1 (Figure appears on preceding page)

An illustrative example of the ecological and evolutionary processes shaping plant microbiota. (a) Plants and their microbiomes occur across large geographic areas that vary in abiotic and biotic environmental factors. Here, we focus on two localities found at the extremes of a temperature gradient. (b) Selection imposed by numerous factors (①–③ in the figure) can shape the diversity and composition of plant microbiota. ① Microbes found in the environment are winnowed during colonization and assemble unique plant-associated microbiomes, a fraction of which are found at high occurrence across host plants and localities (taxa denoted by asterisks in panel b). Moreover, host plant variation across localities as a result of responses to temperature or other environmental factors (indicated by different host plant color in panel b), genetically distinct populations, or different plant species can also shape plant microbiota. ② Geographic location and corresponding environmental features determine the pool of microbes available for plant colonization. ③ Microbe-microbe interactions will likely vary across localities, in turn driving compositional differences in plant microbiota. In addition to selection, plant microbiota are also shaped by (c) drift, (d) dispersal, and (e) evolution. (c) Drift results from stochastic differences in the growth rates of individual plant microbiome members and can cause plant microbiota to compositionally diverge despite exposure to the same environments. Each point in panel c depicts the composition of a sampled plant microbiome, and the distance between two points reflects compositional similarity. Although location has a clear effect on plant microbiota, samples within a locality still exhibit variation despite being collected from the same host plant species in the same environment. Some of this variation will be caused by ecological drift. (d) Dispersal can also contribute to compositional differences observed among plant microbiota across spatial scales. Microbial dispersal can occur over small and large spatial extents, giving rise to compositional differences between pairs of plant microbiota that scale with distance. (e) Evolutionary change occurring in individual plant microbiome members can lead to altered population growth rates and species interactions (as depicted in panel b), both of which could lead to shifts in plant microbiota. In addition to elucidating its role in shaping plant microbiota, research on evolutionary change in the plant microbiome has led to important discoveries of adaptations to a plant-associated lifestyle. For example, genetic differences between plant-associated and free-living relatives reveal microbial adaptations to life with plants (as shown in panel e). However, we propose that microbial adaptation might be occurring to other features of the local plant-associated environment, including host plant variation, the abiotic environment, and resident microbiota (as depicted in ①–③ in panel b). Genomic analysis of core microbiome members across these factors could reveal adaptations that are otherwise hidden by the cryptic diversity within amplicon sequence variants or operational taxonomic units.

diversity and composition (**Figure 1**). New species arise in communities via dispersal and evolution, and their relative abundance is shaped by selection, ecological drift, and continued dispersal (59, 133). Studies testing how selection influences community diversity and composition have dominated plant microbiome research since the inception of the field, but recent work highlights the potential importance of the other three processes.

Selection. Plants are not homogenous microbial habitats. Different plant habitats such as leaves (80), roots (104), or flowers (112) typically harbor unique microbiota (6, 11; but see 89). This is due to variation across plant habitats in plant-derived resources as well as physical and chemical properties resulting from structural differences and exposure to different features of the environment. For example, roots and leaves impose different selection on microbiota due to both their structural differences and exposure to soil versus air, respectively (6, 58). After plant habitat, variation in the abiotic and biotic environment can exert large direct and indirect effects on plant-associated microbiota. Climate-driven geographical variation in soil microorganisms (5) can drive the composition of plant microbiota due to the predominance of horizontal transmission (83). Alternatively, environmental variation, both abiotic (46) and biotic (66), can indirectly shape plant microbiota through plant responses. Interactions among microbes can also have large effects on community composition, in which the presence of particular microbial groups or even single taxa alter the plant microbiome via both antagonistic and beneficial interactions (20, 37, 121, 129, 140). Finally, numerous studies demonstrate that variation within (13, 32) and across (45, 75, 99, 128) host plant species can affect both the diversity and composition of plant microbiota. However, the extent to which variation across host plants shapes microbiota seems to vary across environments (12, 13, 132) and habitats within plants (89, 113).

Ecological drift. Ecological drift—stochastic variation in growth and death—can be a potent driver of community composition under certain scenarios, notably when communities have few

species and exhibit low overall abundance and selection is weak (52, 150). Endophytic communities found within plant organs may be particularly prone to ecological drift due to their low overall abundance compared to epiphytic communities, especially during early plant development (38, 48). Stochastic changes in the relative abundance of individual species within a community can have large downstream effects on community composition when coupled with selection, such as altered interaction strengths among community members. For example, increasing the relative abundance of six randomly chosen members of a soil microbial community led to compositional changes consistent with competitive exclusion between the increased member and close relatives in the community (148). The high levels of unexplained variance in plant microbiome composition and diversity, as well as the abundance of individual taxa, are, at least in part, due to drift (87). Both statistical (123) and experimental (2, 52) approaches will be needed to fully understand and quantify the role of drift in driving composition differences among plant microbial communities.

Dispersal. While selection and drift alter the abundance of existing members within a community, dispersal and diversification are how new species arise in communities. Plant-colonizing microorganisms disperse from the surrounding environment (i.e., horizontal transmission), including soil (83), neighboring plants and interacting animals (111), and the air column (28), although vertical transmission via seed can also occur (100, 119). After initial colonization, microbial dispersal to and from plants is likely to occur. Yet despite its omnipresence, dispersal is a difficult process to quantify; this is especially true for microorganisms. The importance of dispersal is inferred by correlating the compositional similarity between two communities with the physical distance separating them, although distance can be confounded by known or unknown environmental factors. A negative correlation between distance and similarity (i.e., distance-decay) suggests that microbial dispersal limitation may contribute to compositional differences between communities. Distance-decay studies of plant microbiota show that the importance of dispersal as a driver of community composition will vary according to the spatial scale, plant habitat, and microbial taxa under consideration (3, 35, 42, 67, 78, 90). Researchers are also beginning to use experimental approaches to evaluate the importance of dispersal in the plant microbiome. Experimentally reducing dispersal among floral nectar microbial communities increased compositional similarities between communities (131). Experimental studies show that bacterial dispersal in soil is restricted to a narrow taxonomic breadth of organisms, which could have direct impacts on the colonization of plant roots (76, 142). Dispersal can also shape plant microbiota through the introduction of priority effects, whereby the order of arrival among microorganisms can either facilitate or inhibit the success of future colonists (18). Furthermore, changes in environmental variables such as resource availability and pH can alter the strength of priority effects (57).

Evolution. Evolutionary change can shape the growth of individual populations and the interactions between species and can ultimately give rise to new species, all of which can affect community composition (55, 62). Direct evidence of evolutionary change occurring within plant microbiota, let alone shaping community composition, is rare outside well-known symbionts (108). Evolved resistance to phage reduced the ability of a bacterial pathogen to proliferate on its host plant, which in turn led to altered composition of plant microbiota (140). Indeed, the evolutionary outcomes of multitrophic interactions within the plant microbiome may play a much larger role in shaping community diversity and composition than is currently recognized (49, 74, 96). The interaction between evolutionary dynamics and dispersal may also have large effects on plant microbiomes. Conceivably, a beneficial mutation arising in one microbial population could spread via dispersal to others, either within or between plant hosts, potentially altering microbe-microbe interactions and ultimately community composition (94). Although the above examples include evolution by

natural selection, genetic drift occurring within microbial populations could also lead to evolutionary change, with consequences for ecological dynamics.

In summary, although the many forms of selection undoubtedly shape plant microbiota, emerging results hint that ecological drift, dispersal, and evolution contribute to the observed variation in the diversity and composition of plant microbiomes. However, the artificial designation of community boundaries imposed by sampling conventions, such as sampling one root or the entire root system at one time point, overlooks the possibility that well-defined root tissues, individual roots, or both may harbor distinct microbiota that vary over time as a result of the processes described above. Increased temporal sampling (48, 97, 143) and methodological advances that allow for near in situ interrogation of host microbiota at fine spatial scales (120) are likely to yield important insight into the organization of plant microbiota and the ecological processes that structure them. A fuller appreciation of all the ecological processes is necessary for our basic understanding of plant microbiota and the successful deployment of plant probiotics.

Adaptation in the Plant Microbiome

Due to the large effects of selection on the diversity and composition of plant microbiota (see above), it stands to reason that microbes have adapted to plant habitats. Evidence from the fossil record and comparative studies with extant plant species show that plant-microbe interactions played an important part throughout land plant evolution and likely before (41, 56). While well-known symbionts such as arbuscular mycorrhizal fungi and nitrogen-fixing bacteria have a long and ongoing coevolutionary history with plants, adaptation in the rest of the microbiome has only been investigated recently.

Clear evidence of adaptation to a plant-associated lifestyle comes from comparative genomic studies and experiments using transposon insertion mutant libraries. Comparative genomics using taxonomically diverse plant-associated bacteria reveals widespread signatures of adaptation conserved across different plant habitats and host plant species (6, 82). Melnyk et al. (91) applied a similar approach to identify genes associated with the transition from commensalism to pathogenicity in a clade of plant-associated *Pseudomonas* strains. Random barcode transposon-site sequencing (BarSeq), in which individually DNA-barcoded transposons are inserted randomly into a recipient genome, has been used to identify bacterial genes required for plant colonization (29) and evasion of plant innate immunity (85).

In addition to a general plant-associated lifestyle, other environmental features may act as selective forces within the plant microbiome. Host plants are found across diverse localities that vary in temperature, precipitation, and numerous other abiotic and biotic variables, and different host plant species themselves can vary in traits relevant for microbiota, such as the quality and quantity of plant-derived resources and innate immunity. Mounting evidence supports the presence of a core set of microbial taxa that exhibit high occupancy and abundance across localities and host plant species (45, 86, 127). Local adaptation is pervasive in both micro- and macroorganisms (72), and we suspect that members of the plant microbiome could be adapting to features of local environments, including host plant, environmental conditions, and resident microbiota (96, 140) (**Figure 1**).

Current methodologies in census experiments are unable to distinguish different bacterial species or populations, and the conventional taxonomic units (operational taxonomic unit or amplicon sequence variant) harbor cryptic functional and genomic diversity (70). However, methods exist to uncover local adaptation in the plant microbiome using both comparative and experimental approaches. Comparative genomics can detect recently diverged populations of plant-associated microbiota across host or environmental variation (4, 33). Parallel genomic

divergence of microbial populations across replicate, naturally occurring host plants or environmental variation would suggest local adaptation. Microbial populations can also be experimentally evolved (130), such that evolutionary change is tracked across independently evolving lineages experiencing different environments (e.g., host plant identity, environment, resident microbiome composition). Combining a BarSeq library with variation in host plants (85), the abiotic environment, or resident microbiota might help identify potential genetic targets of adaptation in the plant microbiome to local factors. For example, Morin et al. (98) grew an *Escherichia coli* BarSeq library singly or alongside a community of three species and demonstrated that genes required for growth in the presence of a microbial community were different from those required in isolation in the same environment.

Although much is left to discover, adaptation to a plant-associated lifestyle seems to include the ability to navigate the plant immune system and make use of the diverse pool of resources occurring in plant habitats. These findings have helped guide reductionist approaches to understand the mechanisms underlying interactions between plants and their microbiota.

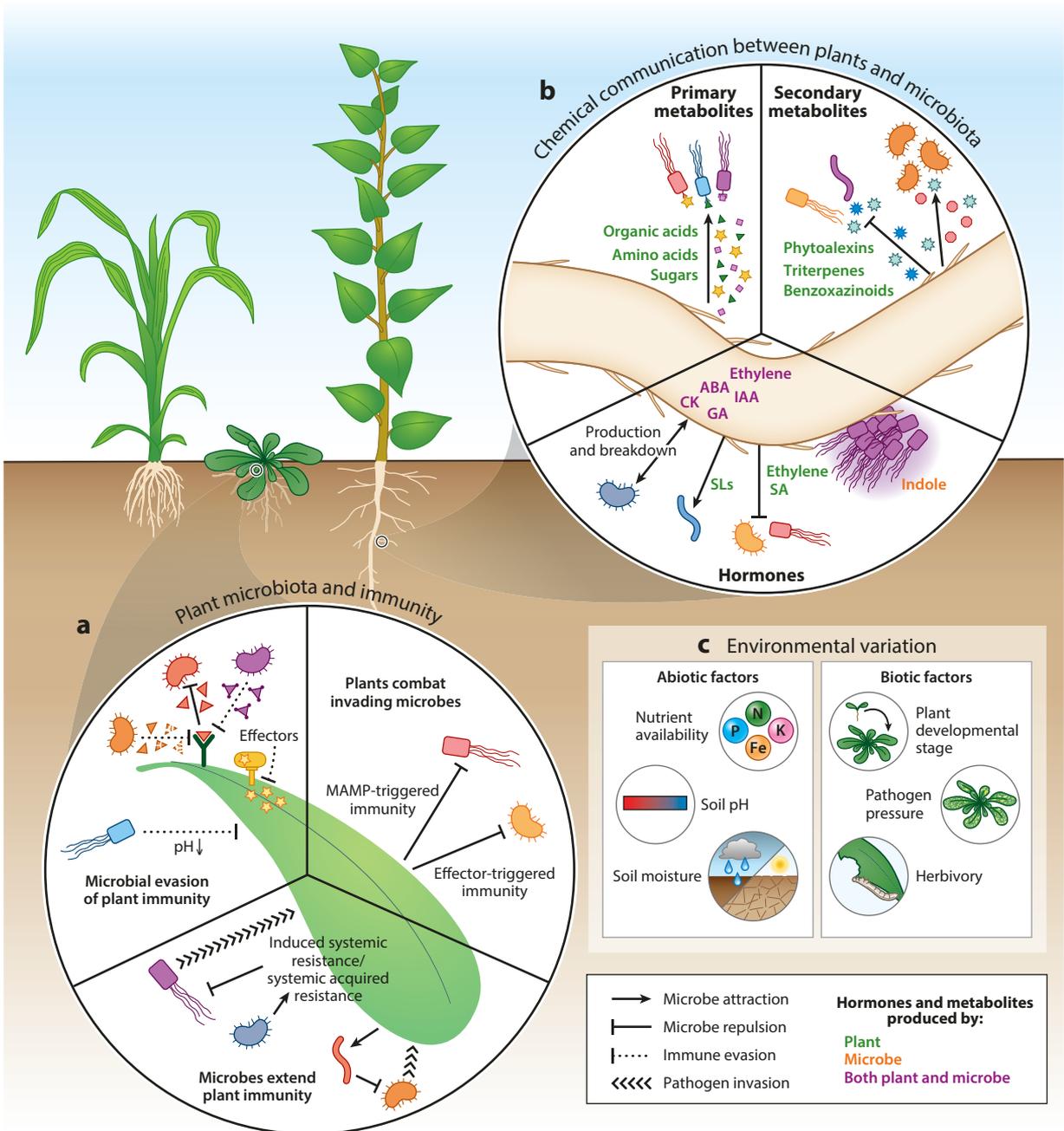
MECHANISMS UNDERLYING THE ASSEMBLY OF PLANT MICROBIOTA AND THEIR EFFECTS ON PLANTS

The stark difference in both diversity and composition between plant-associated microbial communities and those found in the surrounding environment implies the existence of mechanisms that selectively gate access of microbes to plant tissues. Although the host genetic contribution to microbiome assembly is usually low (138), plant-associated microbial communities often change in response to specific metabolic and environmental conditions, including nutrient starvation, drought, and pathogen infection (8, 23, 44, 66, 117). Mounting evidence indicates that the plant microbiome is associated with increased tolerance of plant hosts to both biotic and abiotic stresses (8, 20, 45, 125, 143). Thus, understanding the mechanisms that govern the distribution and abundance of plant-associated microorganisms has become a priority in the plant microbiome field, aided by the development of novel methodology (81, 101, 102, 136, 146). In the following sections, we review the known mechanisms underlying plant microbiome assembly, focusing largely on the role of the plant immune system in maintaining microbiome homeostasis and chemical communication between plants and microbes (Figure 2).

Revisiting the Plant Immune System in the Context of Microbiomes

The plant immune system comprises two tiers of receptors that detect both nonself and modified-self molecules (69). The first tier of immunity is based on plasma membrane receptors that perceive extracellular ligands, including the now well-known microbe-associated molecular patterns (MAMPs), flagellin, peptidoglycan, and chitin. MAMP perception triggers the release of reactive oxygen species, activates phosphorylation cascades, and initiates transcriptional reprogramming and the synthesis of antimicrobial proteins and secondary metabolites. Adapted pathogens, however, produce effector molecules that act within the plant cell or in the apoplast to suppress this layer of immune response and thus promote plant susceptibility. In turn, plants have evolved a second tier of intracellular receptors (namely nucleotide-binding leucine-rich repeat proteins) that perceive the presence of effectors and trigger a stronger immune response (69). Although this model describes the interactions between plants and pathogens, it can also explain how the plant immune system influences microbiome structure (27, 126). Supporting the engagement of plant immunity with nonpathogenic microbes, colonization of *Arabidopsis* by the mutualist fungus *Piriformospora indica* is reduced in mutants that display enhanced responses to MAMPs (68). Interestingly, exaggerated growth and compositional perturbation of leaf endophytic bacterial

communities are observed in *Arabidopsis* mutants with defective MAMP-triggered immunity (MTI) (24), coinciding with poor plant health. Wild-type plants inoculated with the perturbed leaf microbiota collected from MTI-defective plants also displayed poor health (24), indicating that plant immunity not only functions to inhibit pathogens but also contributes to the assembly of a healthy microbiome, which includes both abundance and composition (71).



(Caption appears on following page)

Figure 2 (Figure appears on preceding page)

The mechanisms underlying assembly of plant microbiota and their effect on plant development. The mechanisms illustrated are separated into (a) plant immunity and (b) chemical communication. (c) The listed abiotic and biotic environmental factors influence both mechanisms. In the microbiota and immunity section (a), microbes evade plant immune surveillance by multiple mechanisms: lowering environmental pH, degrading or modifying the activating MAMP, or injecting the plant with immunosuppressive effectors. When pathogenic bacteria are identified by plant immunity, the plant combats the invading bacteria by either MAMP-triggered immunity or effector-triggered immunity. The surrounding microbes can even extend plant immunity by activating the plant's induced systemic resistance and systemic acquired resistance or by directly inhibiting the invading pathogen. Abbreviations: ABA, abscisic acid; CK, cytokinin; GA, gibberellic acid; IAA, indole-3-acetic acid; MAMP, microbe-associated molecular pattern; SA, salicylic acid; SLs, strigolactones.

Microbial colonization in the face of plant immunity. All plant-associated microorganisms, pathogenic and nonpathogenic alike, are confronted with the plant immune system (145). An inspection of a collection of 608 plant-associated bacteria revealed that 97% of them carry at least one potentially immunogenic MAMP (126). This raises the question of how nonpathogenic microbes gain access to host plant habitats when confronted with plant immunity. The answer, in part, is the sophisticated ability of the plant immune system to differentiate pathogenic from nonpathogenic microbes using different combinations of molecular cues at fine spatial scales (149). Furthermore, plants are capable of finely disarming microbial pathogens without perturbing resident microbiota (139). However, accumulating evidence confirms that nonpathogenic (often beneficial) microbes can also suppress MTI, evade MTI, or both during plant colonization (50, 68, 85, 124, 144). Nonpathogenic microorganisms can suppress MTI via MAMP variation, modification, or degradation (126) but also through changes in lifestyle or by altering the surrounding environment to escape detection. Using a library of transposon mutants, Liu et al. (85) found that the genes required for tempering biofilm production in the rhizosphere-associated *Pseudomonas* sp. WCS365 were selected for in the presence of a functioning plant immune system. Additionally, MTI suppression by at least some *Pseudomonas* strains can occur through gluconic acid-mediated lowering of extracellular pH (144). Both symbiotic and pathogenic fungi such as *Laccaria bicolor* and *Magnaporthe oryzae*, respectively, can suppress host defense response through production of effectors that target the host jasmonic acid signaling pathway (105, 107). Thus, similar to what has been described for pathogens, commensal and beneficial microbes may manipulate plant immune responses through a variety of independently evolved mechanisms (Figure 2), most of which remain to be determined. Extending this to a community context yields the notion that plant immune suppression may be a common good because suppression should occur not only for the suppressing organisms but also for the resident community. This raises the question of how spatially restricted microbe-microbe interactions may influence plant immune suppression and subsequent colonization.

Plant microbiota extend plant immunity. The interactions between innate immunity and plant microbiota are not unidirectional; instead, the plant microbiome can also directly and indirectly extend plant immunity (Figure 2). Indirect stimulation by associated microbiota can occur via induced systemic resistance or induced activation, whereby plant microbiota cause the plant immune system to enter either a sensitive or active state, respectively, both of which can lead to increased resistance to pathogenic microorganisms (106, 134). Direct interactions between members of the plant microbiome can also have large effects on resistance to pathogens, independently of the plant's intrinsic immune system. Bacterial inhibition of fungal pathogens seems to be a general phenomenon in the plant microbiome (37, 51) and can arise through complex interactions within bacterial consortia (115, 116). Similarly, antimicrobial production is prevalent among bacterial members of the phyllosphere and likely performs a protective role, too (61). Protection by the phyllosphere microbiota against a bacterial pathogen was abolished under

high nutrient conditions, indicating that microbe-mediated protection can rely on competitive dynamics in addition to antimicrobial compounds (10). Finally, the viral component of the plant microbiome may also play a role in plant defense. A recent study showed that treating bacterial wilt disease in tomato by a combination of phages leads to either reduced pathogen density or selection for slow-growing, phage-resistant mutants, both resulting in decreased disease symptoms (140). These studies raise the exciting possibility that plants may actively enrich particular members of their microbiota (8) or alter their immune vigilance (93) to aid in pathogen defense. These hypotheses remain to be fully tested.

Chemical Communication Between Plants and Microbiota

The plant microbiome is a complex web of species interactions governed, to a large extent, by chemical communication between plants and microbes as well as microbe-microbe communication. Below, we focus on recent advances in our understanding of these dynamic interactions in the context of the plant immune system and plant development.

Immunity. Recent research reveals the role of individual plant immune molecules in fine-tuning microbiome structure. The synthesis of the defense hormones salicylic acid and ethylene alters root microbiota composition in both *Arabidopsis* and tomato plants (79) through the selection of tolerant microorganisms (26). The vast array of secondary metabolites, called phytoalexins, employed by plants to combat invading microbes (1) seem to also have a broader function of shaping the entire community of plant microbiota (14, 63, 64, 125, 135). For example, the tryptophan-derived molecule camalexin is produced by many Brassicaceae species following immune activation and is also capable of inhibiting fungal pathogens and particular bacteria, thereby altering the root microbiome (73). Additional plant-derived metabolites with roles in defense that influence the assembly of the microbiome, promote the attraction or repulsion of specific strains, or both include triterpenes (65), strigolactones (22), and benzoxazinoids (31). In the case of triterpenes, thalianin and arabidin affect the growth of individual bacterial strains and microbiome assembly in *Arabidopsis* (65).

Development. Beyond the immune system, plants and microbes exhibit a complex chemical communication that involves compounds exuded by both plants and microbes. Plant development consists of a multilayered network of interactions between plant hormone levels and environmental cues. As a colonization mechanism, microbes can disrupt this plant developmental network by exploiting hormone production or degradation. To date, most work has focused on the plant hormone auxin, primarily indole-3-acetic acid (IAA), and its role in plant-microbe interactions. Specific plant-derived indole derivatives can modulate *Bacillus subtilis* biofilm formation and plant colonization (47). A variety of plant-associated bacteria can produce auxin- and indole-related compounds, which are intermediates in the IAA biosynthetic pathway of both plants and bacteria (53). In conjunction, bacteria with the IAA catabolic gene cluster (*iac*) can degrade IAA to catechol for use as an energy source, potentially filling a metabolic niche created by other auxin-producing bacteria (36). Within a complex synthetic bacterial community, root development is maintained by the presence of a widespread root-inhabiting bacterial genus, *Variovorax*, through its ability to revert root growth inhibition via auxin degradation (43). Different plant species can harbor microbiota that collectively produce different indole compound profiles, hinting that specificity may exist between plant hosts and the integration of microbiota into their developmental program (53). As well as auxins, a variety of pathogenic and commensal microbes can directly or indirectly produce ethylene and induce fruit ripening or degrade it through production of 1-aminocyclopropane-1-carboxylic acid deaminase, thus promoting root growth (110).

Plants and their microbiota can interact throughout host plant development by way of molecules other than hormones. Plants exude a multitude of compounds into the rhizosphere that affect microbiome assembly, which in turn influences plant health and development (88, 103). Exometabolomic profiling of root exudates over the lifecycle of *Avena barbata* linked substrate preferences, such as amino acids, sugars, and organic acids, of specific microbiome members to their assembly at different plant developmental stages (147). In conjunction, researchers have begun elucidating microbial chemoreceptors and their role in responding to the chemical cues from their plant host (40). Stable isotope probing is another promising approach that has revealed mechanisms of metabolite flow in the plant microbiome (54), including identifying Saccharibacteria, a seemingly ubiquitous uncultured bacteria phylum, as participating in the carbon flow in the *Avena fatua* rhizosphere (122). Other approaches, including imaging high-resolution mass spectrometry, have been used to probe metabolites in the *Arabidopsis* phyllosphere under colonization by commensal and pathogenic bacterial strains (114).

Environmental Variation Shapes the Mechanistic Interactions Between Plants and Microbiota

Plants and their microbiomes must cope with environmental variation, including changing temperature, light, humidity, soil chemistry, and water availability. This environmental variation often leads to shifts in the diversity or composition of plant microbiota (17, 25, 63). While these environmentally induced shifts can be due to direct microbial responses, they are often indirectly caused by plant responses (21), which can shift plant microbiota through the mechanisms described above. For example, in *Arabidopsis*, phosphorus starvation alters the root microbiome through the phosphate starvation response, which is integrated with the immune system through the master transcriptional regulator phosphate starvation response 1 and its effects on the jasmonic and salicylic acid pathways (23, 44, 64, 95). Secondary metabolites produced by plants under various environmental stresses, including iron (125) and phosphate (64) limitation, can also shape the plant microbiome by either selectively enriching or inhibiting particular members. Plant responses to abiotic stress can also be linked with microbiota through shared signaling components, such as salt stress tolerance, which is linked through the chitin receptor, chitin elicitor receptor kinase 1 (39). Plant exudation and bacterial uptake of the metabolite glycerol-3-phosphate were strongly associated with the enrichment of Actinobacteria in the *Sorghum bicolor* root under drought stress (143). Intriguingly, environmentally induced changes in plant-microbe interactions through immune suppression may persist transgenerationally via a so-called thermomemory (84). Moreover, the cross talk between abiotic and biotic environmental responses can change over the course of plant development, in turn shifting the composition of plant microbiota (9). Lastly, insect herbivory can perturb the leaf microbiome through the induction of plant defense responses (66). These examples illustrate that variation in both the abiotic and biotic environment can elicit changes in the plant microbiome through plant responses (**Figure 2**).

The studies reviewed above provide critical mechanistic insight into the assembly and function of plant microbiota. Methodological advances including the use of synthetic communities of genome-sequenced microbial strains, dual omics of host plants and microbiota, and increased resolution of microfluidic and microscopy techniques will be critical for our understanding of the diversity and composition observed in plant microbiota across host plant habitats and environments. An important point is that these mechanisms are proximal, in that they provide genetic explanations, molecular explanations, or both of how the assembly and modulation of plant microbiota occur. However, the ultimate mechanisms, why such assembly and modulation occur, remain unknown. For example, from an evolutionary perspective, the plant immune system

is adaptive because it inhibits the growth of harmful pathogens; whether or not it is adaptive because it can shape the rest of the plant microbiome is unknown. The hypothesis that natural selection has shaped the active control of plant microbiota through their effects on host plant fitness, whether that control be plant immunity, hormones, or exudates, is appealing but need not be the only explanation (7). For example, beyond the acquisition and maintenance of a benign microbiome, plant-mediated modulation of microbiota by way of immunity, hormones, or exudates may be entirely incidental to the innate role of such processes in plant health.

INTEGRATING ECOLOGICAL AND REDUCTIONIST APPROACHES FOR A MORE COMPLETE UNDERSTANDING OF PLANT MICROBIOTA

Both ecological and reductionist approaches have led to important discoveries in plant microbiome research, yet in isolation, these approaches are limited. Mechanism is impossible to discern using amplicon or metagenomic surveys, and ecological importance is often unknown with reductionist approaches. Given the labor, cost, and time associated with large-scale surveys and detailed mechanistic studies, integrating these approaches may not always be feasible in a single research group, let alone a single project. Instead, integration needs to take place across research groups to advance the understanding of the mechanistic underpinnings and consequences of variation observed in plant microbiota in natural and managed habitats.

One exemplary research focus is disease suppressive soils. Disease suppression occurs when resident soil microorganisms limit the occurrence, negative consequences, or both of plant antagonists, including pathogenic fungi and bacteria (118). Specific disease suppression (SDS) is defined by a single or select group of microorganisms inhibiting the growth of a specific plant pathogen (118). Decades of work demonstrated that some forms of SDS, such as take-all decline (the inhibition of the wheat disease caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici*), are globally distributed, follow remarkably similar temporal dynamics, and can be transferred through soil transplants to localities that exhibit non-SDS (77). These ecologically based characterizations eventually led to the mechanistic discovery that take-all decline is caused by the production of the antibiotic 2,4-diacetylphloroglucinol by a select group of soil- and rhizosphere-inhabiting *Pseudomonas* spp. (34, 77, 109). A similar research trajectory, which began with ecological characterizations of a widespread plant-microbial phenomenon followed by targeted reductionist studies, led to the discovery of several nonexclusive mechanisms that contribute to the SDS of the fungal root pathogen *Rhizoctonia solani* in sugar beets (19, 20, 30, 92). In particular, an operative mechanism in the sugar beet root microbial community effectively suppresses *R. solani* infection (20). First, the authors identified biosynthetic gene clusters enriched in the root metagenome of plants growing in suppressive soil with *R. solani*, which included genes involved in secondary metabolite biosynthesis. These gene clusters were associated with several bacterial genera that, when cultured from root samples and reinoculated back onto sugar beet roots, could inhibit *R. solani* infection. Finally, engineered mutants of one of the protective bacterial isolates convincingly demonstrated the genetic basis of the protective function.

While questions remain surrounding the initiation and generality of the SDS dynamics uncovered in the examples above, the general research plan has proved effective: (a) ecological characterization to establish biological importance, (b) targeted reductionist studies to identify mechanism, and (c) evaluation of generality across multiple systems (137, 141). In light of this plan, the plant microbiome field is poised to identify many of the mechanisms that underlie the fundamental and widespread patterns observed in census studies, namely that plants harbor distinct microbiota that can vary across organs, host individuals, and environments (**Figure 3**). Evaluating the generality

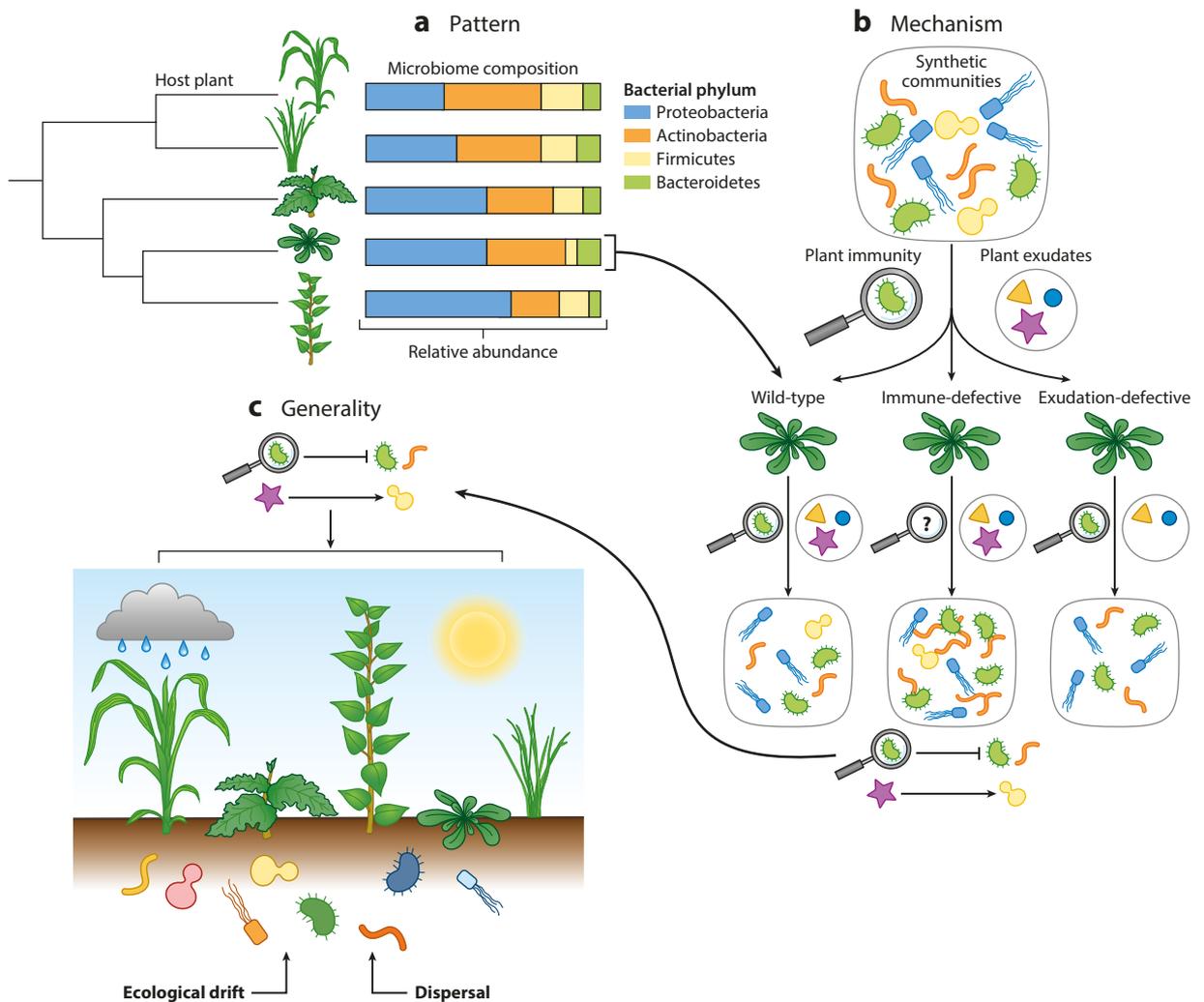


Figure 3

A three-part research plan to advance our understanding of the plant microbiome under natural and managed settings. (a) The plan begins with describing patterns in the distribution of microbial taxa or genes using census experiments. (b) The mechanism or mechanisms underlying these patterns are then investigated using reductionist experiments. (c) Finally, evaluating the operative role of the identified mechanisms across diverse systems demonstrates generality. For example, numerous census experiments reveal that the composition of plant microbiota, including those found in leaves and roots, varies among host plants. Reductionist studies, which use a variety of approaches including synthetic communities paired with targeted mutants of model plant species, support the mechanism that innate plant immunity and plant exudation profiles shape leaf and root microbiota. However, these mechanisms have yet to be evaluated across a wide range of settings, which could include different host plant populations or species, variation in the abiotic and biotic environment, and different resident microbiota from which the plant microbiome is derived. Moreover, the ecological processes of dispersal and drift occurring in conjunction with selection may obscure the operation of the identified mechanisms under more complex settings.

of these emerging mechanisms across biological contexts and linking them with plant health will be the next step toward a more complete understanding of the ecology, evolution, and function of plant microbiomes.

DISCLOSURE STATEMENT

J.L.D. is a co-founder of AgBiome, LLC, a company dedicated to using microbes to improve plant productivity.

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